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Review Article

An update of spectrum and frequency of *GJB2* mutations causing hearing loss in the south of Iran: A literature review

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ABSTRACT

Objective: Mutations in the *GJB2* gene are a major cause of autosomal recessive non-syndromic HL (ARNSHL) in many populations. Previous studies have estimated the average frequency of *GJB2* mutations to be between 16 and 18% in Iran, but would vary among different ethnic groups. Here, we have taken together and reviewed results from our three previous publications and data from search other published mutation reports to provide a comprehensive collection of data for *GJB2* mutations and HL in the south of Iran.

Methods: In all, 447 unrelated families were included and analyzed for the prevalence and type of the *GJB2* gene mutations.

Results: Totally, the frequency of *GJB2* mutations was found to be 11.5% in the southern provinces studied which is significantly lower than that identified in Northern populations of Iran, and also a southwest to southeast Iranian gradient in the frequency of *GJB2* mutations is suggested.

Conclusions: This study highlights the importance of establishing prevalence, based on the local population for screening and diagnostic programs of live births in Iran.

1. Introduction

Hearing loss (HL) is a complex disorder and accounts for 0.2% of newborns worldwide (http://hearing.screening.nhs.uk/nationalprog). Approximately 50–70% of HL being related to genetic causes. It is estimated that 70% of cases includes non-syndromic forms (NSHL), where the hearing deficit is the only sign [1]. Although all Mendelian inheritance patterns have been observed for pre lingual HL, Autosomal recessive mode of inheritance (ARNSHL) makes up 80% of the NSHL cases [2]. ARNSHL is highly heterogeneous, for which over 100 mapped loci are known to be involved (http://hereditaryhearingloss.org). Nevertheless, a single locus, DFNB1 (13q11-12) which contains *GJB2* (NM_004004.5) and *GJB6* (NM_001110219.2) genes, accounts for about 50% of the etiology in many Western populations [3,4]. *GJB2* encodes the connexin-26 (Cx26) gap-junction channel protein that underlies both intercellular communication among supporting cells and homeostasis of the cochlear fluids, endolymph and perilymph [5]. The gene has a simple genomic structure with only two exons, with exon 1 being untranslated. To date, more than 100 pathogenic mutations in the *GJB2* gene and over 4 pathogenic deletions, including gross del(GJB6-D13S1830) and del(GJB6-D13S1854) in the *GJB6* gene have been identified resulting in ARNSHL [5]. The prevalence of *GJB2* mutations varies among different populations [6–9]. In Caucasians, c.35delG is the most common mutation with the carrier frequency as high as 2–4% [10]. However, c.167delT, c.235delC and c.71G > A are the most frequent mutations in the Ashkenazi Jewish [11], Japanese [12] and Indians [13], respectively.

Since the last decade, a series of studies have been conducted on the Iranian population in order to identify the mutation spectrum and prevalence of *GJB2* mutations [14–20]. The diverse ethnicities, coupled with the high rate of consanguinity rates (38% in average) [21] tend to change mutation frequencies among ethnic groups. Therefore, for accurate and effective genetic counseling, studying certain ethnic groups is of high importance [22]. In this paper, we summarized the published

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data on the frequency and profile of the *GJB2* gene mutations in 447 unrelated families from 5 different provinces; namely Khuzestan, Sistan & Baluchestan, Hormozgan, Fars and Bushehr in the south Iran compared to other parts of this country.

2. Materials and methods

This study included results from our four previous publications on *GJB2*-Related HL in Iran. We also performed a PubMed, Google Scholar, and Web of Science search using search terms "*GJB2* mutations" or "connexin 26" and "Iran". Among search results, we limited the search to humans that held information on molecular genetics of HL. Studies were included when fulfilling the following three criteria: (1) performance on non-syndromic hearing loss subjects, (2) described the ethnicity of tested subjects and (3) detected all the *GJB2* mutations. Studies were excluded if hearing loss was a result of environmental factors such as infection, trauma, rubella, meningitis, mumps, ototoxic drugs and premature birth. Research data including 447 unrelated deaf families of southern provinces were collected. The frequency and mutation type of 447 deaf families were extracted from relevant studies and categorized, corresponding with geographical boundaries. *In silico* analyses were also performed by available software tools to predict the pathogenicity of the mutations.

3. Results

Data from 447 unrelated families were gathered for analysis (Fig. 1). The groups studied consisted of 50 family from Fars province (11.2%), 73 family from Khuzestan (16.5%), 184 family from Sistan & Baluchestan (41.2%), 121 family from Hormozgan (27%) and 19 family from Bushehr province (4.1%). The *GJB2* mutation allele frequencies of each studied group included 13, 15.7, 6.2, 3.7, 4.3% of total studied families (n = 447) of Fars, Bushehr, Khuzestan, Hormozgan and Sistan & Baluchestan provinces, respectively.

Totally, 18 different variants were identified, 13 of which were reported as pathogenic. These include: c.35delG, 23+1G > A, c.167delT, c.238G > A, c.71G > A, c.279G > A, c.445G > A, c.507A > C, c.592G > A, c. 82C > A, c.336G > T, c.163A > G, c.100A > G. In the studied populations, c.35delG was the most frequent mutation. The highest rate of c.35delG mutation was detected in Fars province with an allele frequency of 7% while we did not find any c.35delG mutation in Sistan & Baluchestan. Fig. 2 shows the distribution of the identified mutations in the schematic structure of Cx26. A specific combination of *GJB2* mutations types and frequencies were found in different studied provinces (Table 1). A higher *GJB2* mutations diversity (7 type) was observed in Fars province while the lowest diversity identified in Khuzestan (1 type).

4. Discussion

This study reviews the prevalence and type of the *GJB2* gene mutations by means of a literature review and compares 447 deaf families from 5 provinces in the south of Iran. The genetic epidemiology of ARNSHL is very different among populations even from neighboring countries, because of subtle variations in their ethnic composition and because of founder effects [23]. The Iranian population is composed of many different ethnic groups, so it is important to discuss ethnic specific data [24]. Here, the most consistent finding was the reduction for *GJB2* mutations frequency of southwest to southeast Iran. Confirming the northwest to southeast *GJB2* HL gradient throughout Iran, our data indicated a southwest to southeast gradient among Iranian populations with a *GJB2* mutations frequency of 20% for Fars province and 7.7% for Sistan & Baluchestan province. The results obtained from other studies have shown that the mutation frequency of *GJB2* varies between 0 and 35% among different parts of Iran [25]. The study performed by Najmabadi et al. [26] on 664 ARNSHL families indicated that *GJB2* related HL account for 16.7% in Iranian population and c.35delG mutation was the most common *GJB2* mutation (~63.1% of the identified *GJB2* mutations). They also found the highest percentage of *GJB2* related HL in the north and northwest regions (north 38.3% and northwest 22.2%) of Iran has been explained by the founder effect [27]. In another cohort study, Hashemzadeh et al. [28] showed that the frequency of *GJB2* mutations to be 27.5% in the north and Northwest provinces of the Iran, while it was less than 4% in the Southeast region. The observed northwest to southeast *GJB2* HL gradient is further supported by data specific to the southeast and northwest Iran, where the populations are related to the neighboring Oman and Turkey [29,30]. Bonyadi et al. [31] presented that *GJB2* mutations were responsible for about 28% of

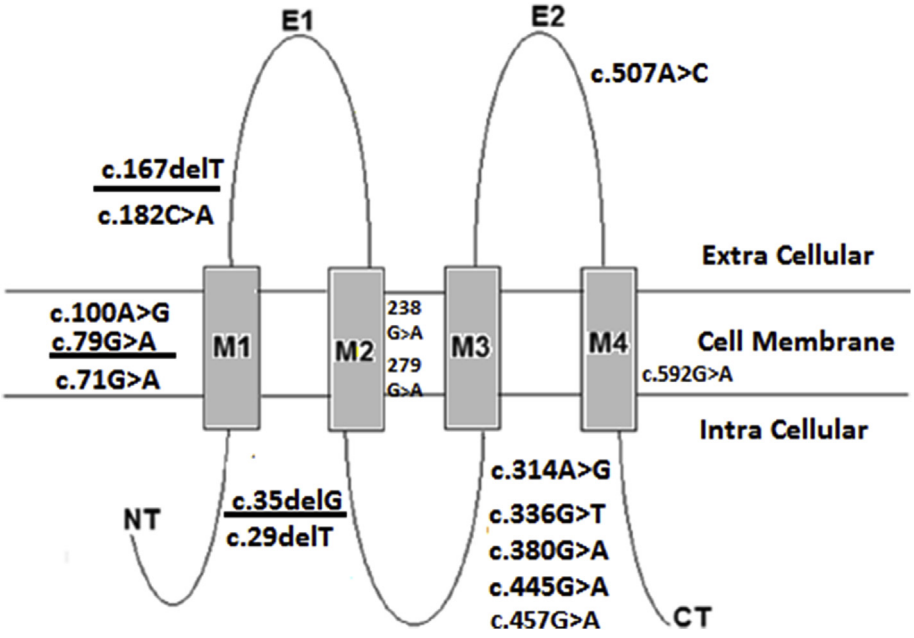

Fig. 1. Schematic structure, domains and distribution of mutations of the Cx-26 protein in this study. The most common mutations in south Iran (c.35delG, c.71G > A and c.167delT) are underlined.

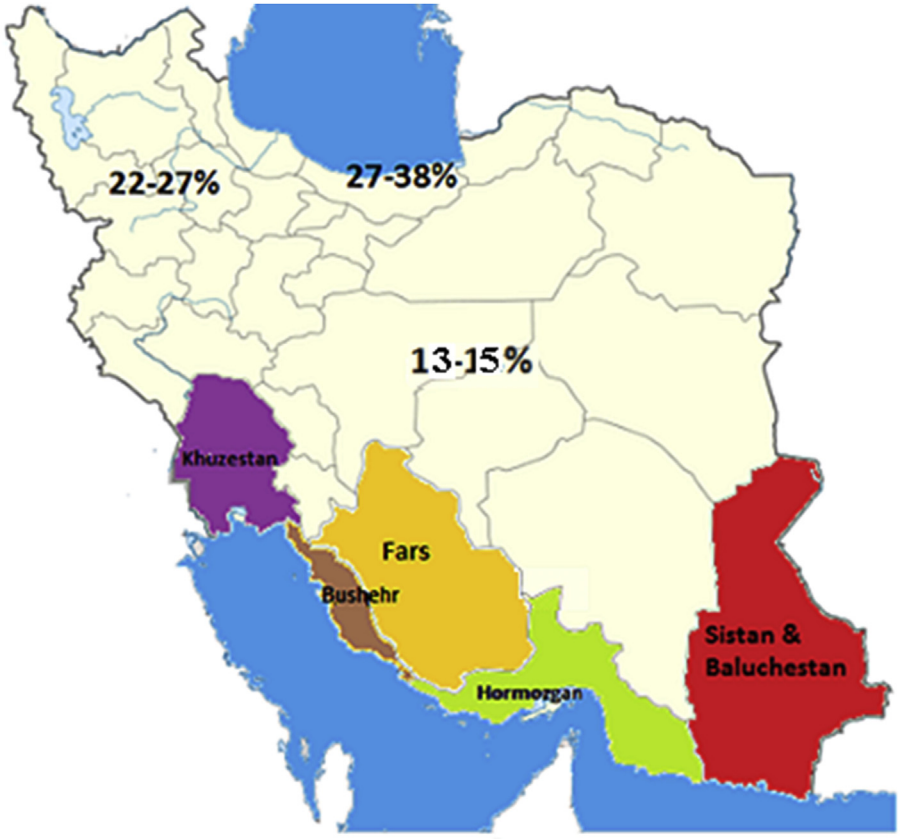

Fig. 2. The prevalence of *GJB2*-related mutations in different region of Iran (northwest 22–27% [26,28], north 27–38% [26,28], center 13–15% [25,26]). Five southen provinces (Khuzestan, Sistan & Baluchestan, Hormozgan, Fars and Bushehr) were shown in the map.

Table 1
GJB2 mutations, their frequencies and *in silico* analyses in five provinces of Iran. The pathogenic mutations and benign variants were separated in the two parts. The mutations were arranged in numerical order. T:Truncated Protein, NT: Non-Truncated Protein, NA: Not Available.

Mutations	No(%)							mutation type	classification	Functional effect	
	Fars	Khuzestan	Hormozgan	Hormozgan	Sistan $ Baluch.	Sistan $ Baluch.	Bushehr			Mutation Taster	SIFT
c.29delT	0	0	0	2(6.25)	0	0	0	Frame shift	T	Disease causing	NA
c.35delG	7(7)	9 (6.16)	3 (1.42)	0	0	0	4 (10.5)	Frame shift	T	Disease causing	NA
c.71G > A	0	0	2 (0.95)	0	2 (1.19)	10 (5)	0	Nonsense	T	Disease causing	Damaging
c.100A > G	1 (1)	0	0	0	0	0	0	Missense	NT	Disease causing	Damaging
c.167delT	0	0	0	0	0	2 (1)	1 (2.63)	Frame shift	T	Disease causing	NA
c.82C > A	1 (1)	0	0	0	0	0	0	Missense	NT	Disease causing	Damaging
c.238G > A	0	0	0	2(6.25)	0	0	0	Missense	NT	Disease causing	Damaging
c.279G > A	0	0	0	0	0	1 (0.5)	0	Missense	NT	Disease causing	Damaging
c.365A > T	1 (1)	0	0	0	0	1 (0.5)	0	Missense	NT	Disease causing	Damaging
c.445G > A	1 (1)	0	0	0	0	0	0	Missense	NT	Disease causing	Damaging
c.507A > C	1 (1)	0	0	0	0	0	0	Missense	NT	Disease causing	Damaging
c.592G > A	1 (1)	0	0	0	0	0	0	Missense	NT	Disease causing	Damaging
23+1G > A	0	0	0	0	0	0	1 (2.63)	Splice site	T	Disease causing	NA
c.79G > A	1 (1)	0	6 (2.85)	0	1 (0.59)	0	0	Missense	NT	polymorphism	Tolerated
c.380G > A	0	0	4 (1.9)	0	2 (1.19)	4 (2)	1 (2.63)	Missense	NT	polymorphism	Benign
c.457G > A	6 (6)	0	3 (1.42)	0	8 (4.76)	0	0	Missense	NT	polymorphism	Benign
c.341A > G	0	1 (0.64)	0	0	1 (0.59)	0	0	Missense	NT	polymorphism	Benign
c.476G > A	0	1 (0.64)	0	0	0	0	0	Missense	NT	polymorphism	Benign
Normal	81	135	192	28	154	182	32				
Total	100	146	210	32	168	200	38				
References	[47]	[48]	[35]	[49]	[28]	[34]	[25]				

ARNSHL in the Iranian Azeri Turkish patients (Northwest Iran) and c.35delG was the most prevalent mutation accounting for 64.5% of *GJB2* mutations, which is similar to the reported results of the Turkish population [32]. Our results showed that the contribution of *GJB2* mutations to ARNSHL is 3.7 and 4.3% in Hormozgan and Sistan & Baluchestan provinces, respectively. This lower rate of c.35delG mutation has been reported in Pakistan population (6.1%) [33]. This finding is comparable to those reported previously by Najmabadi et al. [26]. They reported the lowest percentage of *GJB2*-related HL in the south of Iran.

Another finding of this study was the mutation spectrum of the southeast, which was different from those of the rest Iranian population regions. Naghavi et al. [34] screened 100 ARNSHL families from Sistan & Baluchestan province in Southeast Iran for *GJB2* mutations. They reported that *GJB2* mutations were detected in 7% of the ARNSHL families studied. Interestingly, c.71G > A was the most frequent *GJB2* mutation, while c.35delG was absent in this ethnicity. Results obtained for the carrier frequency of c.71G > A mutation was 40–55% in Baluchi population whereas 1.45% in the rest Iranian groups. However, the Baluchi population is ethnically distinct from the rest of Iran. Besides, 16 unrelated ARNSHL families from Hormozgan province (south of Iran) were reviewed, with a frequency of 12.5% for *GJB2* gene mutations. We didn't find c.35delG mutation in this population. In another study performed by Sasanfar et al. [35] on 105 ARNSHL families from Hormozgan province showed that *GJB2* related HL account for 2.3% in this population and the role of c.35delG mutation was only ~17.6% of the identified *GJB2* variants. This low rate of 35delG mutation has been reported in some populations of Pakistani and Omani families [30,36].

In the study performed by Koohiyan et al. [37] on 40 ARNSHL families indicated that *GJB2* related HL account for 22.5% in center Iran. This is about five times the frequency of *GJB2* mutations in Sistan & Baluchestan province. On the basis of these results and our previous studies [28], it can be concluded that the frequency of c.35delG decreases gradually northwest to center and center to southeast (Fig. 2).

In our studied populations, the most common mutation was c.35delG, accounting for 38.0% of *GJB2* mutations. The c.35delG mutation is found to be the most common mutation in many world populations as well as many countries in the Middle East. It is mostly presented in the Turkey, north and northwest of Iran and it is much less present in southeast of Iran, Pakistan and Arabic countries located in the southern part of Persian Gulf [32,36,38,39]. The analysis of the geographical distribution of mutations located in *GJB2* gene showed more allelic heterogeneity in the north and center compared to the south of Iran [28]. The three most common mutations of the *GJB2* gene in the south of Iran, namely, c.35delG, c.71G > A and c.167delT are responsible for ~75% of all pathogenic alleles in the south Iran (Table 1). The c.35delG mutation, which is the most common (up to 85%) among northern regions [28], makes up for 45% of *GJB2* mutations in the southern populations. The c.71G > A and c.167delT are the second and third common mutations, with a sum of 27.5% and 5.9% of all pathogenic alleles.

The p.Trp24*, a nonsense mutation is the result of c.71G > A transition, changing TGG codon for Trp residue to a stop codon, which leads to a truncated protein with probably no functional properties. *In silico* analyses are consistent with the pathogenicity of the mutation (Table 1). The c.71G > A mutation is the most common mutation in Slovak Romany, Pakistan and Indian populations [40–42]. The rate of carriers of c.71G > A mutation is 4.08% in Pakistan population [36]. This mutation shows a high frequency in Baluchi group (southeast Iran) and accounts for 80% of the mutant alleles in this ethnicity, where the population is related to the neighboring Pakistan [34].

The p.Arg127His, substituting arginine with histidine residue, is the result of c.380G > A transition. Although some researchers have concluded that c.380G > A is only a polymorphism without any association with HL (http://www.crg.es/deafness/). Our *In silico* analyses also confirm it; in contrast of mutation taster software, PolyPhen detected it as a benign mutation. Interestingly, c.380G > A is homogeneously distributed throughout Iran, arginine 127 might be a hotspot point [43]. This review showed a particular combination of *GJB2* mutations diversity in different provinces of south Iran. A higher *GJB2* mutations diversity (7 type) was detected in Fars province, suggesting the co-existence of several different ethnic groups and immigrations to big city such as Shiraz during last century.

More recently, researchers have shown that mutations of *GJB2* can function in a di genic manner with the *GJB6* genes [44]. Hence, mutation analysis of this gene should be considered in *GJB2* heterozygotes. Hashemzadeh et al. showed that more than 30% of patients were heterozygous carrier in south and southeast Iran [35]. Further investigation is needed to detect the genetic cause of HL in the patients with mono allelic *GJB2* mutations [45,46].

5. Conclusions

The critical and specific position of Iran and the existence of various ethnic groups with different cultures suggest the high heterogeneity throughout Iran but specific intra ethnic traditions such as intragroup marriages may give rise to a high homogeneity in some loci and mutations within groups. *GJB2* mutations are responsible for ~11.5% of deaf families in south Iran [28,34,47] that is less than that in northwest region (22–27%) [26,28] showing migration pathway from west to east through the silk route. Regarding the *GJB2* mutations, c.35delG is the most common mutation that is tested first. In studied populations, specific mutations are common, which are detected in each group; for example, the frequency of the c.71G > A shows a high rate in Sistan & Baluchestan provinces, accounting for 75% of the mutant alleles. This study highlights the importance of establishing incidence, based on the local population of specific and common *GJB2* mutations in designing screening strategies.

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